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(54) Title: ISLET CELLS FROM HUMAN EMBRYONIC STEM CELLS

(57) Abstract: This disclosure provides a system for producing pancreatic islet cells from embryonic stem cells. Differentiation is initiated towards endoderm cells, and focused using reagents that promote emergence of islet precursors and mature insulin-secreting cells. High quality populations of islet cells can be produced in commercial quantities for use in research, drug screening, or regenerative medicine.

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**AMENDED CLAIMS and STATEMENT**

**[Received by the International Bureau on 12 August 2003 (12.08.03):  
original claims 1-22 replaced by amended claims 1-22]**

**CLAIMS**

1. An isolated cell population obtained by differentiating primate pluripotent stem (pPS) cells, in which at least 5% of the cells secrete one or more of the following proteins from an endogenous gene: insulin, glucagon, somatostatin, or pancreatic polypeptide.
2. An isolated cell population obtained by differentiating primate pluripotent stem (pPS) cells as part of a system for generating pancreatic hormone secreting cells,  
wherein at least 5% of the cells secrete one or more of the following proteins from an endogenous gene: insulin, glucagon, somatostatin, or pancreatic polypeptide,  
and wherein the system further comprises the line of pPS cells from which the differentiated cells were produced.
3. A system for generating pancreatic hormone secreting cells, comprising the differentiated cell population of claim 1, and the undifferentiated pPS cell line from which it was obtained.
4. The differentiated cell population according to any of claims 1-3, in which at least 5% of the cells express at least two of the following markers: Pdx1, Ngn3, insulin, IAPP, and Nkx6.1.
5. The differentiated cell population according to any of claims 1-3, comprising less than 1% undifferentiated pPS cells.
6. The differentiated cell population according to any of claims 1-3, which when implanted into hyperglycemic NOD mice causes fasting glucose level to drop below 200 mg/dL.
7. The differentiated cell population according to any of claims 1-3, containing cells that have been genetically altered to express telomerase reverse transcriptase (TERT) at an elevated level.
8. The differentiated cell population according to any of claims 1-3, which has the same genome as an established line of primate embryonic stem cells.
9. The differentiated cell population according to any of claims 1-3, wherein the pPS cells are progeny of cells obtained from a human blastocyst.
10. The differentiated cell population according to any of claims 1-3, wherein the pPS cells are human embryonic stem cells.

11. A method for obtaining polypeptide-secreting cells, comprising culturing pPS cells or their progeny in a mixture of islet cell differentiation factors, thereby obtaining a cell population in which at least 5% of the cells secrete at least one of the following proteins from an endogenous gene: insulin, glucagon, somatostatin, and pancreatic polypeptide.
12. The method of claim 11, wherein the pPS cells are differentiated to form embryoid bodies, or cells with characteristics of hepatocytes or gut endothelium.
13. The method of claim 11, wherein the mixture of islet cell differentiation factors comprises at least one of the following: activin A, nicotinamide, cyclopamine, betacellulin, and IGF-1.
14. The method of claim 11, wherein the mixture of islet cell differentiation factors comprises a TGF- $\beta$  antagonist and one or more mitogens.
15. The method of claim 11, further comprising genetically altering the cells to cause expression of a pancreatic transcription factor, such as Neurogenin 3.
16. A method for producing insulin, glucagon, or somatostatin, comprising:
  - a) culturing a differentiated cell population according to any of claims 1-3, and
  - b) harvesting insulin, glucagon, or somatostatin secreted by the cultured cells.
17. A device for the production of insulin, glucagon, or somatostatin, containing:
  - a) a differentiated cell population according to any of claims 1-3, and
  - b) a semipermeable membrane that prevents passage of the cell population, but permits passage of insulin, glucagon, or somatostatin secreted by the cell population.
18. A pharmaceutical composition, comprising the differentiated cell population according to any of claims 1-3.
19. A method of screening a compound for its ability to modulate islet cell function, comprising combining the compound with a differentiated cell population according to any of claims 1-3, determining any phenotypic or metabolic changes in the cell population that result from being combined with the compound, and correlating the change with an ability of the compound to modulate secretion of insulin, glucagon, or somatostatin.
20. A method of reconstituting islet cell function or treating Type 1 diabetes in an subject, comprising administering to the subject a differentiated cell population according to any of claims 1-3.

21. Use of the differentiated cell population according to any of claims 1-3 in the preparation of a medicament for treatment of a condition associated with deficiency of insulin, glucagon, or somatostatin.
22. The use according to claim 21, wherein the condition is Type I diabetes.

**STATEMENT UNDER ARTICLE 19 (1)**

The amendments to the claims are supported throughout the specification and claims as originally filed.

In particular, new claims 2 and 3 are based on claim 1 and claim 10 as originally presented, page 3 lines 8-15, and page 21 lines 3-11 of the specification. No new matter is introduced into the disclosure as a result of entering these amendments.